REMARKS

Claims 118-183 are presently pending in the application and stand rejected under 35 U.S.C. § 112, first paragraph (enablement) and under the judicially created doctrine of obviousness-type double patenting. Applicants apologize for any confusion caused by listing claims in their previous response. Claims 118-183 are pending and remain unaltered by this submission.

35 U.S.C. § 112, first paragraph

Applicants acknowledge with appreciation that the rejection of claims 118-183 as allegedly non-enabled for methods of using a zinc finger protein with two or more regulatory domains has been withdrawn in view of the arguments presented in the response and declaration by Adreas Reik, filed 12 February 2003. (Supplemental Final Office Action, paragraph 4).

Nevertheless, the rejection of claims 118-183 as allegedly not enabled for methods in which a ZFP polypeptide is introduced has been maintained. (Supplemental Final Office Action, paragraphs 6-8). The arguments, declaration and additional evidence filed February 6, 2003 and June 12, 2003 were deemed unpersuasive.

Because a *prima facie* case of non-enablement has not been established and because the Office has improperly deemed all the evidence proffered by Applicants in regards to enablement as "unpersuasive," Applicants again traverse the rejection.

A *prima facie* case of non-enablement has not been established

For the reasons of record, Applicant submits that the Office has failed to establish a *prima facie* case of non-enablement. As previously discussed, enablement is a fact-dependent inquiry that can be facilitated by using the standards articulated in *In re Wands*. Indeed, the situation in *Wands* is highly analogous to that at hand. In *Wands,* the Federal Circuit held that claims to generic monoclonal antibodies were enabled by a specification that taught the entire procedure of making monoclonal antibodies. Moreover, in view of the high level of skill in the art and routine nature of each step of the antibody-making procedure, the court held that the amount of experimentation required to make other monoclonals was extensive, but not undue.

The Examiner asserts that the specification fails to provide specific guidance or working examples regarding delivery of proteins. *See,* page 4 of Office Action. The assertion that the specification does not provide specific guidance is incorrect and unsupported by any reasoned

argument. Applicants have repeatedly pointed to specific passages of their specification that provide specific guidance for delivering zinc finger proteins to cells. *See, e.g.*, pages 43-47 of the specification and in the references cited in this text. Indeed, like the applicant in *Wands*, Applicant has established that the specification at issue teaches how one would go about introducing proteins (ZFPs) into cells, as set forth in the claims. Moreover, Applicants remind the Office that working examples are never required in order to satisfy the enablement requirement. All that is required is that the specification set forth sufficient teachings to allow one of skill in the art to practice the claimed subject matter. In the pending case, following the guidance set forth by Applicants, a skilled artisan could readily practice the claimed methods. Thus, for the reasons previously of record and those reiterated herein, Applicant again submits that the specification fully enables the claimed methods.

Rebuttal Evidence has not been properly considered

Even assuming, for the sake of argument only, that a *prima facie* case of nonenablement had been established, extrinsic evidence (including an expert declaration and Abstracts) submitted by Applicant rebutting this rejection has not been properly considered by the Office.

With regard to declaratory evidence of record, Applicants note that the Office has erroneously dismissed Dr. Pabo's Declaration pursuant to 37 C.F.R. § 1.132 on the grounds that it "expresses opinion without justification by evidence or reasoning." (Supplemental Final Office Action, paragraph 7). The Office has totally misrepresented Dr. Pabo's declaration, in which every conclusion Dr. Pabo reaches is based on evidence and/or sound scientific reasoning. It is well settled that statements of opinion presented in Declarations made by qualified persons of ordinary skill in the art must be considered. *In re Alton,* 37 USPQ2d 1578 (Fed. Cir. 1996). In *In re Alton,* the Federal Circuit also commented that they were "aware of no reason why opinion evidence relating to a fact issue should not be considered by an Examiner." 37 USPQ2d 1578 at 1583 n10. *See, also, Ex parte Obukowicz*, 27 USPQ2d 1063 (BPAI 1993).

Dr. Pabo clearly established that he possessed significant and relevant expertise in the fields of protein delivery generally and in zinc finger proteins in particular at the time Applicants' specification was filed. Indeed, as noted in his Declaration, Dr. Pabo is a co-inventor on several issued U.S. patents in the area of protein delivery. (*See,* Pabo Declaration, ¶2). The issue date of all but one of these patents pre-dates the filing date of Applicants' specification. Dr. Pabo also demonstrated that he had vast experience in the area of zinc finger proteins. Not only is Dr. Pabo well qualified to opine on the specification at issue but, in addition, his opinions are based on years of fact and data gathering experience.

Dr. Pabo's fact- and reasoning-based conclusion that Applicants' disclosure fully enables one of skill in the art to practice the claimed methods was also made after examining the specification, as filed. In reaching this conclusion, Dr. Pabo considered several facts, including the fact that membrane translocation peptides, toxins, liposomes, antibodies and other moieties described on pages 43-47 of the specification had been used to introduce proteins into cells for some time. (Pabo Declaration, ¶7). Dr. Pabo found the teachings of the specification to be "extensive" and also concluded that "a skilled worker would have easily recognized that the protein delivery moieties described in the specification and known in the field could be used to deliver an engineered ZFP to a cell." (Pabo Declaration, ¶7). Thus, contrary to the Office's assertion, Dr. Pabo based his conclusion on sound scientific facts including his own vast knowledge and the teachings of the specification.

In addition, Dr. Pabo reached his conclusions after considering references available to the public at the time of filing, including his own patents regarding tat peptides for protein delivery and the Debs and Phelan references. Despite Dr. Pabo's Declaration, the Office has continually dismissed these references. Debs is discredited because it "uses only in vitro cultured cells, and shows only regulation of expression by a sensitive assay from a transfected reporter gene vectors ... [and does not show] use of an engineered zinc finger protein." (Supplemental Final Office Action, paragraph 7). In addition, Debs is alleged to teach away from modulation of endogenous genes. *Id.* For its part, Phelan was alleged not to describe delivery of extracellular proteins to cells, but rather "spreading" of proteins from cell to cell. *Id.*

Applicants again note that the question of enablement at issue in this case is not whether endogenous genes can be regulated by zinc finger proteins *in vivo* (which Applicants have clearly shown they can). Rather, the question is whether zinc finger proteins can be administered to a cell and whether they would be functional. Both references are directly pertinent to this inquiry -- Debs by teaching that a transcription factor delivered to a cell was functional and Phelan for teaching that an HSV VP22 peptide can mediate entry into a cell. The so-called distinction between "delivering" and "spreading" made by the Office in regard to Phelan does not change the fact that this reference used VP22 translocation domain of HSV (as described on page 44, line 23 of the specification) to introduce a functional protein into a cell. The particulars about the nature of the transcription factor or its target are not at issue here and both Debs and Phelan are entirely on point with regard to the issue of protein delivery.

Dr. Pabo concurred that Debs and Phelan are relevant to the question of what the specification teaches one of skill in the art regarding predictability of protein delivery techniques (and their applicability to ZFPs). Indeed, Dr. Pabo concluded that both Debs and Phelan "plainly

confirm that protein delivery vehicles described in the specification had been successfully employed thereby confirming that protein delivery as a whole was **not** unpredictable as of January 1999." (Pabo Declaration, ¶ 8). Again, Dr. Pabo's conclusion that protein delivery was not unpredictable and that the ZFPs would function when delivered as protein is based on cogent scientific arguments, buttressed with references to relevant scientific literature available to the public as of Applicants' filing date, including Dr. Pabo's own demonstration evidenced by his ow issued patents that tat peptides as disclosed in the specification (*e.g.*, page 44, lines 16-18) are able to mediate transport of a protein into a cell.

In addition to the Pabo Declaration, Applicants provided still further evidence (Yeh et el. Abstract) that functional ZFPs as claimed can be delivered as proteins. (See, Exhibit A of Response filed June 12, 2003, Yeh et al.). As with Dr. Pabo's declaration, the Office has deemed Yeh unpersuasive, this time on the grounds that it (1) is irrelevant to the instant enablement inquiry because it is a post-filing date reference; (2) is not an enabling reference; (3) demonstrates that the "prior art has not previously shown peptide-mediated delivery of transcription factors;" and (4) teaches away from the use the antennapedia domain. (Supplemental Final Office Action, paragraph 8). Applicants address each contention in turn.

Although publications filed after the filing date generally cannot show enablement in and of themselves, such evidence is probative if it is not offered to supplement the disclosure of the application, but rather as evidence "that the disclosed [methods] would have been operative." *Gould v. Quigg,* 3 USPQ2d 1302, 1035 (Fed. Cir. 1987). Here, Yeh evidences that the disclosed methods are indeed operative and that protein ZFPs can be delivered to, and are functional in, cells.

Applicants also submit that Yeh is a sufficiently enabling reference. The fact that Yeh is an Abstract and does not set forth methods in detail does not effect its relevance to the pending enablement inquiry. Yeh does not need to provide blueprint-type details of how the protein was delivered because such techniques were known in the art and set forth in the specification as filed. Moreover, Yeh does in fact provide all that is necessary for the skilled artisan to practice their methods -- methods of making fusion proteins, introducing these proteins and assaying mRNA activity are all routine to one working in the field. Thus, Yeh provides still further evidence that protein delivery was not unpredictable in view of the teachings of the specification and that it would not have required undue experimentation to practice the claimed methods using proteins.

Turning to the allegation that Yeh is "first" demonstration of peptide-mediated delivery of a transcription factor, Applicants again note this is inaccurate. When read in context of the

Abstract as a whole, Yeh is actually narrowly referring to the "first" *in vivo* demonstration of use of an artificial transcription factor to upregulate VEGF-A endogenous gene expression. In fact, as noted above, techniques for delivering proteins to a cell were known at the time of filing and described in the specification as well as in Dr. Pabo's declaration (and references cited therein).

Furthermore, even if Yeh was the first time that anyone had actually demonstrated delivery of functional engineered ZFPs, this in no way means that the specification was not enabling as filed. The test is whether the specification teaches one how to practice the claimed methods, not whether working examples are actually provided. Thus, Yeh is simply further evidence that the teachings of the specification are sufficient to enable one of skill in the art to practice the claimed methods.

Finally, Applicants note that Yeh in no way indicates that the antennepedia domain does not function. Rather, Yeh teaches that this domain may not be as efficient as other peptides for delivering engineered ZFPs. Indicating that various techniques work with differing levels of efficiency is far cry from establishing that one is inoperative. Furthermore, the notion that one of ordinary skill in the art must have reasonable assurance of obtaining positive results on every occasion has been emphatically rejected (*In re Angstadt*, 190 USPQ 214, 219 (CCPA 1976)). So long as it is clear that some species render the claims operative, the inclusion of some possible inoperative species does not invalidate the claim under paragraph 1, of 35 U.S.C. §112. (*In re Cook*, 439 F.2d 730, 735, 169 USPQ 298, CCPA 1971; *Horton v. Stevens*, 7 USPQ2d 1245, 1247, Fed. Cir. 1988).

Case Declaration

In addition, to further rebut any argument that using the claimed methods involving proteins *per se* is "unpredictable" or would require "undue experimentation," Applicants submit herewith Declaration by Dr. Casey Case documenting that, using the methods set forth in the specification, a skilled artisan could readily practice the claimed methods using proteins themselves. Dr. Case has worked in this field and it was under his supervision that constructs encoding zinc finger proteins were prepared and supplied to the authors of the Yeh paper. (Case Declaration, ¶4).

Dr. Case's Declaration also submits data establishing that zinc finger proteins are functional when administered as proteins:

> 4. As the data depicted in Figures 1-6 of Exhibit B shows, experiments have been conducted demonstrating that engineered zinc finger proteins (ZFPs), delivered in protein form, modulate endogenous VEGF gene

expression *in vitro* and *in vivo*. These experiments were summarized in Yeh, which was previously made of record in this case. We at Sangamo BioSciences provided polynucleotides encoding engineered ZFPs targeted to VEGF genes used for these experiments. As shown in Figure 1 of Exhibit B, standard site-directed insertional mutagenesis techniques were used to subclone a variety of internalization peptide sequences (IS) into the 5' end of Sangamo constructs. As shown in Figure 2, a functional domain (Activation) was subcloned onto the 3' end of the Sangamo constructs. Thus, the exemplary construct shown in Figure 2 encodes a fusion protein comprising a histidine tag sequence (HIS), an internalization peptide sequence (EP), a nuclear localization signal (NLS), a VEGF-ZFP (ZFP DNA binding) and a functional domain (Activation). Also shown in Figure 2 is the experimental protocol for making these constructs and expressing the resulting fusion proteins. The protocols correspond essentially to the techniques set forth on pages 33-36 of the specification. Yeh et al. used various peptide internalization sequences (IS), including sequences selected by phage display (EP or PPD) and antennapedia (AP), as described on page 44 of the specification. ZFPs and ZFP-IS fusion proteins were delivered *in vitro* or *in vivo* by standard techniques, for example as described in the last paragraph of page 49 of the specification. The effect of the ZFPs on VEGF-A mRNA levels was measured after time, using mRNA detection assays, essentially as described on page 37, lines 14-21 of the specification.

5. Figures 3-6 of Exhibit B show that VEGF expression was enhanced *in vivo* and *in vitro* upon a single application of ZFP-IS fusion proteins. In particular, Figure 3 of Exhibit B shows the results of an *in vitro* experiment, and demonstrates that an internalization sequence mediates transport of ZFPs into cells. Figure 4 shows the large percent increase in VEGFA mRNA expression *in vitro* after administration of VEGF-targeted ZFP fusion proteins. Figure 5 is a graph depicting transduction efficiency of a VEGF-ZFP-IS fusion protein, in which the internalization sequence used is either a phage display selected peptide (EP) or antennapedia (AP). Figure 5 demonstrates that <u>both</u> EP and AP internalization sequences significantly increase the levels of VEGF mRNA in cells. Figure 6 of Exhibit B shows *in vivo* activation of VEGF-A by two different ZFP-IS fusion proteins (IP1-VEGF ZFP and IP2-VEGF ZFP) after injection into a mouse hindlimb skeletal muscle. The levels of VEGFA mRNA increased at least 200% *in vivo* after administration of ZFP-IS proteins. Thus, using the VEGF-targeted ZFPs provided by Sangamo Biosciences, it was demonstrated that direct fusion protein transduction is an effective way to upregulate VEGF-A transcription *in vitro* and *in vivo*.

This data demonstrates that engineered ZFPs can be used to modulate expression of endogenous genes *in vitro* and in living animals when delivered as proteins. (Case Declaration, ¶6). In addition, a variety of protein delivery mediators (*e.g.*, PPDs, antennapedia, and the like) can be used and, because ZFPs can be designed and/or selected to bind to any predetermined

sequence, methods similar to those described in his declaration with regard to delivery of VEGFA-targeted ZFPs in protein form are equally applicable to any endogenous gene of interest. (Case Declaration, ¶6). Indeed, Dr. Case agrees with Dr. Pabo's conclusion as set forth in the Pabo Declaration, namely that, as a technical matter, a skilled worker could have readily delivered ZFPs in protein form to cells in view of the teachings of the specification, together with the common general knowledge, tools and methods available as of the effective filing date of January 1999. (Case Declaration, ¶7).

In sum, even though a *prima facie* case of nonenablement has not been established, Applicants have provided ample factual evidence demonstrating that the specification enables the pending claims throughout their scope and withdrawal of the rejection is respectfully requested.

35 U.S.C. § 103(a)

Applicants note with appreciation that all the rejections under 35 U.S.C. § 103(a) have been withdrawn. In paragraph 5 of the Final Office Action, it is stated that ". . . one of skill in the art would expect the unmodified exogenous zinc finger proteins of Liu '96 to operate by regulation of gene transcription because at the time of filing of the instant application the prior art, for example Choo *et al.* and Liu *et al.* '97, provided clear teachings that zinc finger proteins acted by modulation of gene transcription."

Applicants disagree with this statement for the reasons of record. In particular, Applicants note that the pending claims recite contacting an engineered zinc finger protein with a target site in an endogenous cellular gene. As pointed out by Dr. Case at the personal interview in parent application 09/229,037 on August 29, 2002, the presence of a binding site for a binding protein within a particular nucleotide sequence does not ensure that the binding protein will bind to that site in a gene; *i.e.*, when the nucleotide sequence comprising the binding site exists within the context of cellular chromatin. Thus, the teaching of an EGR-1 binding site in the TGF-β1 gene sequence by Liu '96 is not a teaching of the binding of EGR-1 to an endogenous cellular gene.

For these reasons, Applicants respectfully maintain that Liu et al. '96 neither teaches nor suggests a zinc finger protein contacting a target site in an endogenous cellular gene (as claimed), let alone an engineered zinc finger protein contacting a target site in a gene.

The Supplemental Final Office Action also contains the following statement:

> The argument that the prior art did not show regulation of endogenous genes by engineered zinc finger proteins is persuasive because, as pointed out by the applicants in their response, the prior art such as Choo et al. and Liu et al. '97 showed use of reporter constructs as substrates for engineered zinc finger proteins. The use of reporter constructs in the prior art served to teach away from the use of endogenous genes as targets of action of engineered zinc finger proteins. The cited prior art does not provide motivation to use an endogenous gene as a target of action of an engineered zinc finger protein with a reasonable expectation of success. (Supplemental Final Office Action, paragraph 9).

Applicants again clarify that, in their response, it was stated that the references disclose regulation of several types of non-endogenous genes, of which a reporter gene is but one type. For example, Choo *et al.* discloses regulation of a chromosomally integrated heterologous cDNA comprising portions of two different genes. *See*, for example, Applicants' Response dated February 4, 2003 at pages 5 and 6.

Obviousness-Type Double Patenting

The Office again asserts that double patenting exists as between certain of the instant claims and those of copending applications 09/229,037 (now U.S. Patent No. 6,534,261); 09/478,681 (now U.S. Patent No. 6,607,882); and 09/897,844. (Supplemental Final Office Action, paragraphs 15-17).

Applicants submit the appropriate terminal disclaimers and fee herewith.

CONCLUSION

Applicants believe that the claimed subject matter is fully enabled in light of the teachings of the specification, and evidence of record (including declaration by Drs. Pabo and Case). If any issues remain to be addressed, the Examiner is encouraged to telephone the undersigned at (650) 493-3400.

Respectfully submitted,

Date: October 30, 2003

By: _____
Dahna S. Pasternak
Registration No. 41,411
Attorney for Applicants

ROBINS & PASTERNAK LLP
1731 Embarcadero Road
Suite 230
Palo Alto, CA 94303
Tel: (650) 493-3400
Fax: (650) 493-3440